

6. Tang X, Shigematsu H, Bekele BN, Roth JA, Minna JD, Hong WK, Gazdar AF, Wistuba, II. EGFR tyrosine kinase domain mutations are detected in histologically normal respiratory epithelium in lung cancer patients. *Cancer research* 2005;65(17):7568-72.

M15-03 Genetics and Epigenetics in Lung Carcinogenesis, Thur, Sept 6, 10:30 - 12:00

Molecular changes in invasion, tumor progression and metastasis

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Despite major improvements in patient management, the prognosis for patients with lung cancer remains dismal. From biological, histopathologic and clinical perspectives, lung cancer is a highly complex neoplasm probably having multiple preneoplastic and tumor progression pathways. Non-small cell lung cancer (NSCLC) represents nearly 80% of lung tumors, and the major histological types are squamous cell carcinoma and adenocarcinoma. Both tumor types are believed to arise after a sequential progression of pre-malignant lesions, including bronchial squamous metaplasia and dysplasias for squamous cell carcinoma, and peripheral atypical adenomatous hyperplasia for a subset of adenocarcinomas.

As our knowledge of the molecular basis of lung cancer pathogenesis, progression and metastasis has increased, new molecular abnormalities and novel targets for therapeutic interventions have been identified. After decades of separate but not equal investigation efforts, prevention and therapy are beginning to converge at the level of early-phase clinical testing. This highly beneficial convergence has encouraged the investigation of known and novel molecular abnormalities at a) progression and invasion of lung preneoplastic lesions, b) tumor progression phenomenon (locally early vs. advanced tumors) and, c) metastasis process. Although multiple lung cancer-related molecular pathways have been recognized to be involved in those lung cancer stages, there is no extensive information of their involvement in the multistep development and progression of lung tumor. Using a comprehensive molecular pathology approach, we are currently investigating the role of several key molecularly abnormal pathways in the early pathogenesis, tumor progression and metastasis of lung cancer, including tyrosine kinase receptors, epithelial mesenchymal transition (EMT), angiogenesis and stem cells markers.

Our recent studies indicate that multiple molecular pathways are differentially dysregulated in the early pathogenesis of the major types of lung cancer, including, among others, inflammation-related changes, inactivation of multiple tumor suppressor genes, and the activation of multiple tyrosine kinases, particularly receptor tyrosine kinases. Recently, using a molecular mapping strategy, we have characterized the EGFR molecular abnormalities in the early pathogenesis of lung adenocarcinomas, and demonstrated that the activation of the nuclear factor kappa B (NF- κ B) and the insulin-like growth (IGF) factor axis are frequent phenomena in the early progression of squamous cell carcinoma of the lung. In addition, our unpublished data suggest that the EMT phenomenon also plays an important role in lung cancer early pathogenesis.

The molecular events involved in the local progression of lung cancers have not been well studied. These studies require the application of molecular pathology-based mapping strategies to study the molecularly abnormal clonal expansion of tumor cells. Our recent studies on EGFR molecular changes (mutation, copy number and protein expression) and in the immunohistochemical expression of multiple markers suggest that molecular heterogeneity is present in lung cancer specimens, and

they need to be further studied to understand the local progression of lung tumors.

Although the mechanisms involved in lung cancer metastasis are not well understood, it is believed that biologically heterogeneous tumors contain subpopulations of cells with different invasive and metastatic properties. To produce metastasis, tumor cells must complete a series of selective and sequential steps. Our recent findings indicate that primary tumor and brain metastasis share multiple molecular abnormalities; however, they differ in the pattern and characteristics of some molecular changes, including HER family receptors and ligands expression. On the other hand, recent studies indicate that to produce brain metastasis, tumor cells must reach the vasculature of the brain, attach to the microvessel endothelial cells, extravasate into the parenchyma, proliferate, and induce angiogenesis. Although there are currently major efforts in the identification of lung cancer stem cells markers, there is no data available on the role of these subpopulation of malignant cells in the development of lung cancer metastasis.

During the last decade, encouraged by the development of methodologies for isolation of cells from small histologic lesions, such as laser microdissection, combined with techniques to perform genomic studies from minute amount of DNA, RNA and protein, several groups have made substantial progress on unveiling the molecular and genetic abnormalities of lung cancer precursor lesions, tumor progression and metastasis, including those evolving to centrally located squamous cell carcinoma and peripheral adenocarcinoma. The recent development of a panel of human normal bronchial epithelial cells immortalized, which can be modified with a combination of oncogenes activation (EGFR, KRAS) and tumor suppressor (TP53) gene knockdowns for in vitro discovery work, coupled with the development of more relevant lung cancer animal models and new high-throughput genomic and proteomic profiling techniques that can be applied to small amount of microdissected tissues may stimulate the scientific community to perform innovative investigations in the fields of molecular and pathology research.

Session M16: Lung Cancer in Women

M16-01

Lung Cancer in Women, Tue, Sept 4, 10:30 - 12:00

Role of biology and genetics in lung cancer in women

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Within a period of less than one hundred years, lung cancer has become the leading global cause of death among men and subsequently women. The new millennium was ushered by frightening statistics of rapidly evolving incidents and mortality from this disease. The role of increased as well as changing pattern of tobacco use was shown to be a cause of many diseases, and in particular, lung cancer.

The accelerated rate of incidents of lung cancer and the decreasing ratio between men and women raised the issue of increased susceptibility of women to this disease. Many case controlled studies suggested that indeed, women might be more susceptible to develop lung neoplastic process. On the other hand, other cohort studies tend to refute this notion. In view of significant coexistence and association of chronic obstructive pulmonary disease and lung cancer, questions are being

raised as to whether there is common genetic susceptibility connection between the two diseases, considering the risk associated with tobacco smoking or independent of it.

Similarly, there is a growing amount of data pointing to differences between the sexes, regarding the pattern of disease, its morbidity, the response to interventions or therapy, and mortality from primary lung cancer. The task of present and future research is to elucidate answers to these questions on the basis of different biology and genetics.

A certain degree of confusion was introduced through indiscriminate and sometimes inappropriately interchanged uses of the terms sex and gender. Both gender and sex linked factors relate to risk of developing lung cancer and therapeutic implications.

In gender related risk factors, smoking plays a major role, probably in girls and boys as well as in adult women and men. In both youth and adulthood, the type of tobacco used as much as the way of smoking play a role. There are however other risk factors, including environmental tobacco smoke, cooking fuels and fumes, employment, environmental pollution, and nutrition. Each of these might interact also with sex-linked factors.

The sex linked factors will generate biological differences on one hand, and genetic ones, on the other.

The biological differences are rooted mostly in reproductive hormones. Several factors have to be considered in risk assessment like the onset of menarche, length of menstrual cycle, and use of hormone replacement therapy. Estrogens are probably playing a role in lung tumorigenesis at many levels. They may act as ligands to estrogen receptors activating cellular proliferation pathways. They may also undergo metabolic activation to reactive intermediates resulting in formation of DNA adducts or cause oxidative damage. Estrogen receptor beta (ER-beta) is the primary receptor expressed in lung tumors and studies suggest its activation influences lung tumor cell proliferation. On the other hand there no sex difference in expression of this receptor which suggests that this might not be dependent on the level of endogenous estrogens. Interaction between the ER-beta and Epidermal Growth Factor Receptor (EGFR) might have therapeutic implications. Each and every one of these, however, is also dependent on the age of the subject as well as smoking status and interaction of the inhaled substances on the hormonal metabolism. These complicated data then, should be taken into account when planning recruitment and sample size of a given study.

Sex-linked factors also influence the genetic differences between men and women. Women are more likely than men to carry various genetic mutations which, similar to biological differences, will be reflected in risk assessment, increased susceptibility to develop lung cancer on one hand, and response to therapy on the other. Here, once more, the increased risk for women can be influenced by tobacco smoking effects through nicotine and metabolism of other carcinogens and their interaction with hormonal effects.

Two classes of enzymes are crucial in the metabolism of tobacco-related carcinogens-the phase I and phase II enzymes. The phase I enzymes responsible for activation of carcinogens to reactive intermediates are counterbalanced by phase II enzymes having a salutary effect of converting these reactive intermediates to inactive conjugates which then can be eliminated. The toxic intermediates bind to the DNA forming detectable DNA adducts. Increased levels of DNA adducts were found in women with lung cancer then in men. This increase in adducts was correlated with expression of cytochrome P450s (CYP1A1) and glutathione-S- transferases (GST) polymorphism associated with metabolic activity of detoxification (phase II) enzymes and also induc-

tion of p53 mutations (higher G-to-T transversion). The p53 mutations then contribute to perpetuation of DNA damage. Among lung cancer in never smokers a different pattern of mutations of p53 was found in men and women.

There appears to be also a pivotal role of DNA adducts on K ras mutation which are more prevalent (after controlling for tobacco effects) in women and associated predominantly with adenocarcinomas.

Series of various genetic mutations are considered as putative factors in increased carcinogenesis or development of specific type of lung cancer. Other genetic factors are potentially responsible for variability in DNA repair mechanism and decreased recovery from DNA damage as well as sensitivity or resistance to chemotherapy or other responses to therapeutic modalities.

The removal of damaged DNA segments or repair of mismatched nucleotides is controlled by a complex family of proteins. Recent studies suggest that the capacity of repair of the DNA is lower in younger patients with family history of lung cancer and in women. Paradoxically also this reduced repair might be responsible for development of less resistance to platinum based chemotherapy since the effects of platinum is through formation of DNA adducts leading to arrest of proliferation cycle and apoptosis. Studies demonstrating reduction of DNA repair suggest better outcome and less development of resistance to therapy.

EGFR mutations (primarily in exons 18, 19, 21) are associated with increased response rate to tyrosine kinase inhibitors (TKIs). The rate of mutations decreases with smoking they are apparently expressed more in women of East Asian origin where there are also reports of increased therapeutic responses. At the same time the toxicity from treatment with EGFR inhibitors (neurosensory, nausea, vomiting, alopecia and neuro-psychiatric) was recorded to be higher in women than men. Bronchoalveolar carcinoma in non smokers is diagnosed more frequently in women than men and women also show better response to therapy with TKI inhibitors.

The association between male-female ratio and survival from lung cancer has been better documented than the association of the ratio with incidence of the disease. Overall women's stage-for-stage improved survival applies to all histological types of lung cancer. Recent study documenting improved survival in women 65 and older with stage I and II lung cancer even when untreated suggests that female lung cancer has different natural history and potentially different tumor biology. Further extensive studies are required to elucidate in women with and without exposure to tobacco the biological and genetic role in relative risk for developing lung cancer, natural history of the primary and metastatic malignancy and potential therapeutic advantages.

M16-02

Lung Cancer in Women, Tue, Sept 4, 10:30 - 12:00

Risk factors for non-smoking women

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Approximately 10% to 15% of lung cancer patients are never-smokers. Lung cancer in never-smokers (LCINS) is more frequently diagnosed in women than in men. Toh et al evaluated 883 NSCLC patients diagnosed in Singapore between 1999 and 2002. Of 286 never-smokers, 68.5% were women compared with just 12% women among current and former-smokers. However, the fact that there is a significantly greater proportion of never-smoker women than there are men may well contribute to the higher incidence of LCINS observed among women. SCLC and squamous cell carcinoma are extremely uncommon